Comparison of the effects of new folkloric hemostatic agent on peripheral nerve function: an electrophysiologic study in rats

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Objective. The aim was to evaluate the effects of a new folkloric medicinal plant extract on peripheral nerve function compared with oxidized regenerated cellulose (OC) and bovine collagen (BC).

Study Design. Under ketamine anesthesia a total of 40 male Sprague-Dawley rat right sciatic nerves were identified. Animals were randomly divided into 5 groups: OC, BC, ankaferd blood stopper (ABS), and negative and positive control groups. The recordings of nerve potentials were carried out using an electrophysiologic data acquisition system. After the application of substances, the nerve conduction velocity (NCV) was recorded for immediate (30 min), early (120 min), and delayed (3 wk) effects on nerve function.

Results. Statistically, differences were not found among the hemostatic agents (OC, BC, and ABS) at baseline and all tested periods (early, immediate, and delayed; $P > .05$). The positive control group exhibited lower NCV values compared with the other solutions at the 30-minute period ($P < .05$) as well as the other tested time periods ($P > .05$). OC exhibited NCV values closer to the positive control group at 120 minutes ($P > .05$).

Conclusions. Folkloric medicinal hemostatic agent could be considered as an acceptable hemostatic material without resulting in any serious peripheral nerve function alterations. The possible desirable effects of bovine collagen and undesirable effects of oxidized cellulose on peripheral nerve function should not be overlooked. (Oral Surg Oral Med Oral Pathol Oral Radiol 2013;115:e1-e6)

Hemostasis is very important in oral and maxillofacial surgery, especially in procedures resulting in open wounds that expose connective tissue. To obtain appropriate surgical area, hemostatic agents are used by oral and maxillofacial surgeons.[1-4] Several commercially available agents have been designed for this purpose. They are manufactured from plants (e.g., cellulose), animal products (e.g., collagen), and synthetic polymers (e.g., alkylene oxide copolymers).[1,2,5]

These agents are placed almost adjacent to the peripheral nerves as a result of local anatomy. After locally implementing these agents in the maxillofacial surgical area, nerve function problems have been reported.[2,3,5] The amount of the nerve damage could be related to the surgical procedure technique or the properties of the inserted hemostatic agents.[2,3,19] Moreover, little data are available in the literature about the nerve function problems after inserting these agents in the surgical area.[2,3,5]

The folkloric medicinal plant extract named Ankaferd Blood Stopper (ABS; Ankaferd Saglik Urunleri, Istanbul, Turkey) has been introduced for bleeding control in clinical health sciences.[4] ABS consists of a standard mixture of the plants *Thymus vulgaris*, *Glycyrrhiza glabra*, *Vitis vinifera*, *Alpinia officinarum*, and *Urtica dioica*. Several in vivo and in vitro studies showed that ABS was an effective hemostatic agent which increased the new bone formation in early bone healing period.[4] Moreover, different studies have documented the effectiveness of this agent in dental practice.[4,8,9,15]

ABS is a traditional folk medicinal plant extract product that has been approved in the management of external hemorrhage.[4] ABS produces hemostatic actions by providing the encapsulated protein network for vital physiologic erythrocyte aggregation.[8] It has been effectively used in periodontal surgery and teeth extrac-

Statement of Clinical Relevance

A folkloric medicinal hemostatic agent may have no adverse effects similar to bovine collagen and oxidized cellulose on peripheral nerve function.

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Received for publication Apr 22, 2011; returned for revision Oct 17, 2011; accepted for publication Oct 24, 2011.

2212-4403/$ - see front matter
doi:10.1016/j.oooo.2011.10.025

tions,[9] bleeding control in gastrointestinal disorders,[10,11] superficial and deep abdominal lacerations,[10,12] mediastinal bleeding,[13] hemophilia A,[14] and von Willebrand disease.[15] ABS has antimicrobial, antiinflammatory, antiatherosclerotic, and antitumor activities that also increases new bone formation in the early bone healing period.[4,16-18]

On the other hand, there is no information about the effect of ABS on peripheral nerve function compared with the other commercially available agents. The present study aimed to evaluate the immediate, early, and delayed effects of ABS compared with different hemostatic agents on peripheral nerve function.

MATERIALS AND METHODS

Animals

Forty male Sprague-Dawley rats weighing 282-335 g (mean wight 297 g) were randomly assigned to 5 groups of 8 rats per group. Animals were obtained from Karadeniz Technical University Research Center. Animals were housed under a 12:12-hour light-dark cycle (light on at 7:00 a.m.) and room temperature of 20 ± 2°C. They were given free access to food and water. Every effort was made to minimize animal suffering and the number of animals used. Experimental procedures were approved by the Animal Experimentation Ethics Committee of Karadeniz Technical University. All experiments were carried out according to local guidelines for the care and use of laboratory animals and the guidelines of the European Community Council for experimental animal care.

Forty animals were divided into 5 groups: oxidized regenerated cellulose (OC; Surgicel; Ethicon, Neuchatel, Switzerland), bovine collagen (BC; Lyostypt; Braun, Melsungen, Germany), ABS, negative control (0.9% NaCl), and positive control (Maxicaine Fort; 80 mg articaine HCl, 0.020 mg epinephrine; VEM, Istanbul, Turkey).

Surgical procedure

The animals were anesthetized with an intraperitoneal injection of ketamine (100 mg/kg body weight), and additional doses were given as needed. The lateral aspect of the right thigh, hip, and flank was then routinely prepared, including trimming off the hair and antisepsis. The sciatic nerve was exposed through a posterolateral longitudinal straight incision going down from the greater trochanter to the lateral condyle of the femur, followed by blunt dissection between the gluteus superficialis and biceps femoris. The entire length of the nerve was made visible and its 3 main distal branches, the common peroneal, tibial, and sural nerves, were carefully identified in the popliteal fossa.

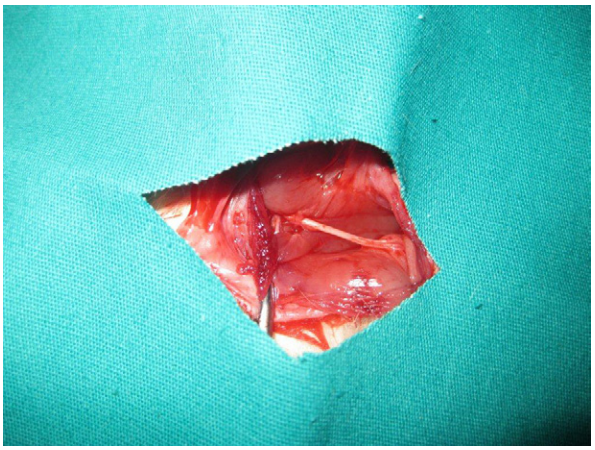

Fig. 1. Exposed rat sciatic nerve.

About 2 cm of the sciatic nerve was exposed above its trifurcation without injuring the epineurium (Figure 1).

Electrophysiologic techniques

The following experiments were performed according to previous study (Alkan et al., 2007).[3] The recordings of nerve potentials were carried out using an electrophysiologic data acquisition system (PowerLab 16/30; AD Instruments, Castle Hill, Australia). Bipolar hook electrodes were used to stimulate the left sciatic nevre and to record the nerve potentials. For the first stimulating point, a hook electrode was placed proximally to the sciatic notch. The recording electrode was placed distally to the sciatic nerve. After stimulating at the first point, the electrode was moved to the second stimulating point, ~1.2 cm distal to the first point (Figure 2). Supramaximal stimuli (10 V) were delivered to the sciatic nerve for 0.2 ms duration from a stimulator on a PowerLab 16/30 data acquisition system. The responses were amplified with an amplifier (ML136 Animal Bioamp; AD Instruments) and stored on a computer. Software (Scope v3.9.1; AD Instruments) was used for data capture and analysis. The latencies were measured from the stimulus artifact to the onset of first wave deflection. Nerve conduction velocities (NCV) were calculated by dividing the distance between stimulating sites by the average latency difference between the onsets of the compound action potential (CAP). The amplitude of the CAP was measured from peak to peak. To determine the latency, electrical stimulation was repeated 10 times and averaged per rat.

After recording the baseline values (for all groups), the nerve was surrounded by OC or BC which was left in the area for 30 minutes, then removed, and NCV values were calculated then and again at the 120-minute period. The procedure was repeated for the 3-week period. In the ABS group, 2 mL ABS was used with a